

The University of Chicago

STUDIES IN CHOLESTEROL METABOLISM

- I. THE EXPERIMENTAL PRODUCTION OF GALLSTONES
WITH A REVIEW OF THE LITERATURE**
- II. EFFECT OF VARIOUS ANESTHETICS ON THE CHOLESTEROL AND SUGAR CONTENT OF THE BLOOD**

**A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF PATHOLOGY

**By
CORNELIUS A. HOSPERS**

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EXPERIMENTAL PRODUCTION OF GALLSTONES

WITH A REVIEW OF THE LITERATURE

CORNELIUS A. HOSPERS, PH.D.

CHICAGO

The origin of biliary concretions is still a disputed question. One avenue of information lies in the experimental production of calculi, and numerous attempts have been made to produce them.

Blachstein¹ (1891) injected *B. coli* and *B. typhosus* intravenously into rabbits, and found that those living from eight to one hundred and nine days "usually" contained in the gallbladder opaque yellowish particles consisting of epithelial cells, leukocytes, amorphous masses of bile pigment and clumps of bacteria. He could detect no differences between the effects of *B. coli* and those of *B. typhosus*. Welch² could not find these particles in the gallbladder of a rabbit four months after injection of *B. typhosus*.

Marcantonio³ reported that he infected a dog's gallbladder, but found no formation of calculus after six months. Injection of lactic acid into the gallbladder of a dog gave negative results. He succeeded in producing a kind of gallstone, however, by deposition of pigment calcium on a foreign body placed in the gallbladder of a dog. This deposit contained no cholesterol.

Gilbert and Dominici⁴ produced cholecystitis in a rabbit by intravesical injection of *B. typhosus* and found a small concretion in the gallbladder.

Mayer⁵ placed pieces of ivory, clay balls and bits of agar in the gallbladders of dogs. After one year, the agar had disappeared, but the solid bodies in some cases had been covered with a thin layer of green-brown or black-green substance, which was pigment calcium and was devoid of cholesterol.

Labes⁶ introduced into the gallbladders of dogs irritating, infective, alkaline and acid substances. Of these, the small or soft bodies were

From the Department of Pathology of the University of Chicago.

1. Blachstein, A. G.: *Bull. Johns Hopkins Hosp.* **2**:96, 1891.

2. Welch, W. H.: *Bull. Johns Hopkins Hosp.* **2**:121, 1891.

3. Marcantonio, A.: *Riforma med.* 1892, vol. 8; cited in Schmidt's *Jahrb.* **238**:13, 1893.

4. Gilbert, A., and Dominici, S. A.: *Compt. rend. Soc. de biol.* **23**:1033, 1893.

5. Mayer, Jacques: *Virchows Arch. f. path. Anat.* **136**:561, 1894.

6. Labes, in Naunyn, B.: *A Treatise on Cholelithiasis*, translated by A. E. Garrod, London, The New Sydenham Society, 1896.

expelled, while the larger or harder ones remained in the gallbladder but never became incrustated. Gallstones placed in the viscus dissolved and ultimately disappeared.

Gilbert⁷ found a stone in the gallbladder of a dog previously inoculated with *B. coli-communis*.

Mignot⁸ was unable to produce stones in the gallbladders of dogs and guinea-pigs by the introduction of foreign bodies. Injection of attenuated strains of *B. coli* resulted in three small biliary calculi in one guinea-pig. He also tamponed the gallbladder of a dog, in the presence of colon bacilli, for one month, after which the tampon was removed. Fourteen months later, the viscus contained from seven to eight faceted stones. These he called cholesterol stones without reporting any analysis.

Richardson⁹ injected agglutinated cultures of *B. typhosus* into the gallbladder of a rabbit and succeeded in producing a firm, brown calculus. No analysis of the stone was reported. The same use of an ordinary bouillon culture of *B. typhosus* in another rabbit failed to produce a concretion.

Cushing¹⁰ produced stones in a similar manner, except that he added another factor, intentionally traumatizing the gallbladder by firmly squeezing it between the fingers for some time after the inoculation. He also produced stones up to 3 mm. in diameter by injecting *B. typhosus* into the ear vein of a rabbit accompanied by trauma to the gallbladder. According to Cushing, these stones were composed chiefly of bile pigments combined with calcium. Some of them were coated with cholesterol.

Miyake,¹¹ working with dogs, was unable to produce stones by infection of the gallbladder with *B. coli* by tying of the cystic duct or by introduction of foreign bodies into the gallbladder, although the last method resulted in incrustation of the glass pearls or particles of granite with bile pigment. The introduction of foreign bodies into the gallbladder along with infection of the organ gave this same incrustation. Ligating the cystic duct in the presence of an infection with *B. coli*, in a dog, resulted in the formation of two faceted stones after nine months. Foreign bodies in the gallbladder plus infection of it, with ligation of the cystic duct, led to the formation of small stones of calcium, cholesterol and pigment in two dogs. One year after infecting the

7. Gilbert, A.: Arch. gén. de méd. **2**:257, 1898.

8. Mignot, R.: Arch. gén. de méd. **2**:129, 1898; Recherches expérimentales et anatomiques sur les cholécystites, Thèse, Paris, 1896.

9. Richardson, M. W.: J. Boston Soc. M. Sc. **3**:79, 1898-1899.

10. Cushing, H.: Bull. Johns Hopkins Hosp. **10**:166, 1899.

11. Miyake, H.: Mitt. a. d. grenzgeb. d. Med. u. Chir. **6**:479, 1900.

gallbladder of a rabbit with *B. coli* and narrowing the cystic duct by placing a piece of gauze under it, Miyake found in the gallbladder a stone which he reported to be rich in cholesterol and containing pigment and much calcium.

Italia¹² reported the production of concretions by intravesical injection of attenuated cultures of *B. typhosus* and *B. coli* into the gallbladders of both dogs and rabbits. The calculi resembled human stones on both gross and microscopic examination, and chemically were shown to contain all the elements found in human stones.

Carmichael¹³ inserted a pebble into the gallbladder of a dog and three weeks later injected *B. typhosus* into the organ. After a month, he found a crystalline deposit of calcium carbonate on the pebble. Glass beads left in the gallbladders of rabbits for four and one-half months were covered by hard, sterile masses composed chiefly of carbonate of lime, protein material and bile pigments. A piece of pith, however, unaccompanied by infection, produced no change. Pith infected with either *B. coli* or *B. typhosus* became surrounded by a curdy mass of carbonates and protein material. None of these foreign body coatings contained cholesterol.

Klinkert¹⁴ repeated Cushing's experiments, injecting avirulent typhoid bacilli into the ear vein of the rabbit. In three of nine rabbits gallstones developed composed of organic substance and pigment calcium without cholesterol.

Chalatow¹⁵ fed cholesterol to rabbits in daily doses of from 0.2 to 1.5 Gm. in from 3 to 25 cc. of sunflower seed oil, for from ten to one hundred and forty-two days. Five of the eight fed showed no evidence of formation of stones. The gallbladder of one rabbit contained some sand of crystalline cholesterol after twenty-six days. Another, after eighty-one days, contained considerably more of this sand. In a third, which lived one hundred and forty-two days, he found this same sand and a "large" crystalline concretion resembling a human cholesterol calculus, but without analysis.

Aoyama¹⁶ tied the cystic duct in fourteen rabbits and in three guinea-pigs. He discovered cholesterol concretions in all but six animals. Eight rabbits and three guinea-pigs received four injections of 0.3 Gm. of cholesterol or cholesterol esters in 1 cc. of ether, subcutaneously every other day. The cystic duct was then ligated. Cholesterol concretions were found in all the animals. Feeding of cholesterol by

12. Italia, F. E.: Policlinico, 1901; cited, Zentralbl. f. Chir. **28**:693, 1901.

13. Carmichael, E. S.: J. Path. & Bact. **8**:453, 1903.

14. Klinkert, D.: Berl. klin. Wchnschr. **48**:335, 1911.

15. Chalataw, S. S.: Beitr. z. path. Anat. u. z. allg. Path. **57**:85, 1913.

16. Aoyama, T.: Deutsche Ztschr. f. Chir. **132**:234, 1914.

stomach tube daily for from eight to ten days in four rabbits also produced cholesterol stones. The character of the stones was established by the crystalline structure, by the solubility in chloroform with reprecipitation in the form of cholesterol crystals, and by the positive color reactions for cholesterol in a chloroform solution of the stones.

Rosenow¹⁷ reported that dogs and rabbits surviving for a long time intravenous injection of streptococci isolated from the gallbladders of patients with cholecystitis not infrequently showed beginning formation of gallstones. He reported later¹⁸ that of eighty animals so treated, six rabbits and three dogs showed definite formation of minute black gallstones, with cholecystitis found constantly. No further description of these stones was given.

Greig¹⁹ found that of eighteen rabbits dying after a long course of intravenous injections of cholera vibrios, nine showed gallstones that, he said, on examination were found to be made up of cholesterol.

Emmerich and Wagner²⁰ exposed the gallbladders of rabbits and injected 0.5 cc. of a twenty-four hour culture of typhoid bacilli in sodium chloride solution from two to three weeks after immunizing the animals with three doses of typhoid serum. Their animals lived from one hundred to three hundred and forty-one days after operation. Cholecystitis resulted in almost every case, and calculi, measuring from pinhead size to 0.5 cm. in diameter, were found in five of sixteen rabbits. These concretions were considered to be of organic material only, since they burned without residue, showed only a slight cholesterol reaction and gave no pigment reaction.

Dewey²¹ injected emulsions of cholesterol intraperitoneally and intravenously into rabbits. One animal receiving 10 cc. of a 2 per cent emulsion intraperitoneally three times a week for forty injections showed no formation of a stone, while another under the same treatment for fifty-two injections showed a few small concretions in the gallbladder. A third received the same injections daily for fifty-eight injections. The gallbladder was filled with small and larger stones. Two rabbits receiving from 5 to 10 cc. of a 1 per cent emulsion, in one case three times a week for sixteen doses and in the other case daily for thirty doses, showed no formation of stones. The combination of thirteen daily intravenous injections with ten daily intraperitoneal injections resulted in the formation of numerous small concretions. Two rabbits received a few intravenous injections followed by a long resting period

17. Rosenow, E. C.: *J. A. M. A.* **63**:1835, 1914.

18. Rosenow, E. C.: *J. Infect. Dis.* **19**:527, 1916.

19. Greig, E. D. W.: *Indian J. M. Research* **3**:259 and 397, 1915-1916.

20. Emmerich, and Wagner, G.: *Centralbl. f. allg. Path. u. path. Anat.* **27**:433, 1916.

21. Dewey, K.: *Arch. Int. Med.* **17**:757, 1916.

and then received a few intraperitoneal injections. The gallbladder of one was negative for stones, while that of the other contained numerous small concretions. The gallstones were irregular, brittle, not faceted and black. Sections of them examined microscopically showed them to be made up of a framework of disintegrated cells held together by pigment and calcareous substances, forming parallel trabeculae. Chemical examination was not reported.

Venema²² made typhoid carriers of rabbits by injecting into the gallbladder one-half loop of a twenty-four hour typhoid agar culture in 0.5 cc. of physiologic solution of sodium chloride. In one animal, he found a soft, bright yellow concretion from 3 to 4 mm. in diameter, which was not analyzed.

Sotti and Torri²³ reported that in 1913 they found sterile concretions of definite morphology in the gallbladders of rabbits after splenectomy and ligation of the ductus choledochus. The chemical structure of the stones was not reported.

Iwanaga²⁴ repeated Aoyama's work. Ligation of the cystic duct, with or without feeding of cholesterol, caused the formation of an amorphous mass in the gallbladder not similar to a gallstone. Subcutaneous injections of cholesterol had no influence on the cholesterol content of the bile and did not cause the formation of these amorphous structures.

Meyer and his co-workers,²⁵ by intravenous and cystic injection of typhoid bacilli, made rabbits chronic carriers. In the gallbladders of the animals that survived for from one hundred to eight hundred and sixteen days were found, invariably, yellowish-green biliary concretions or blackish calculi, consisting of lime salts with traces of bile pigment and organic material, but with no cholesterol. No calculi were found in the gallbladders of the rabbits living less than thirty days, but they were found in all animals surviving the infection one hundred days or longer.

Badile²⁶ infected the gallbladders of dogs with *B. coli* and *B. typhosus* after narrowing the ductus choledochus in each with a ligature. Cholecystitis resulted, but there was no evidence of formation of stones. Narrowing the duct and placing gauze or silk thread in the gallbladder caused only slight precipitation on the foreign body. When a fine cholesterol emulsion was introduced into the dogs' gallbladders after the duct in each had been narrowed, small cholesterol

22. Venema, T. A.: *Berl. klin. Wchnschr.* **54**:815, 1917.

23. Sotti, G., and Torri, O.: *Pathologica* **12**:369, 390 and 423, 1920.

24. Iwanaga, H., cited in *Centralbl. f. allg. Path. u. path. Anat.* **33**:191, 1922.

25. Meyer, K. F.: Neilson, N. M., and Feusier, M. L.: *J. Infect. Dis.* **28**:456 and 510, 1921.

26. Badile, L.: *Pathologica* **15**:307, 1923.

concretions formed. These stones were fragile and gave positive reactions for bile pigment and cholesterol. Microscopically, they showed an amorphous structure inclosing cholesterol granules.

Rous et al.²⁷ discovered incidentally that small calcium carbonate and calcium bilirubinate stones were formed along the glass and rubber tubings after intubating the gallbladders of dogs for the collection of bile under sterile conditions.

Agrifoglio²⁸ loosely ligated the common bile duct in dogs, and then injected colon and typhoid bacilli intravenously or into the gallbladder. Concretions formed only in those animals in which attenuated typhoid bacilli had been injected directly into the gallbladder.

Engel and Cserna²⁹ repeated some of Dewey's experiments. They injected a 2 per cent emulsion of cholesterol daily into the peritoneal cavities of seven rabbits for from four to ten months but were unable to produce gallstones in any case.

Fujimaki³⁰ observed that in rats fed for a long time on a diet deficient in vitamin A there developed, in sequence, bladder, renal and bile duct calculi. The biliary calculi consisted mainly of cholesterol and pigment. Neither lack of vitamin B nor restriction of the protein seemed to be concerned in such formation of stones.

Stern³¹ found no cholesterol coagulation in the gallbladders of rabbits after ten daily intravenous injections of cholesterol.

Hansen³² fed eight rabbits cholesterol in oil in doses of from 0.5 to 1 Gm. daily for from five to seventy-seven days. In four, cholesterol crystals of microscopic size developed in the gallbladder. One, fed 0.5 Gm. for twelve days, showed three pinpoint-sized pearls of cholesterol. A rabbit that received 10 cc. of a 1 per cent emulsion of cholesterol intraperitoneally once daily for sixty days also showed the cholesterol crystals. Another rabbit that received this same solution subcutaneously, intraperitoneally and intravenously once daily for sixty-six days showed clumps of cholesterol in the gallbladder. The injection of this solution intraperitoneally daily for fifty-six days into a rabbit, which then received cholesterol by stomach tube for eighteen days, resulted in the formation of many pigment kernels and cholesterol crystals in the gallbladder. By narrowing the cystic duct of the gallbladder with a ligature in otherwise normal rabbits, Hansen produced

27. Rous, P.: McMaster, P. D., and Drury, D. R.: *J. Exper. Med.* **39**:77, 1924.

28. Agrifoglio, M.: *Clin. med. ital.* **55**:89, 1924.

29. Engel, K., and Cserna, S.: *Wien. klin. Wchnschr.* **38**:123, 1925.

30. Fujimaki, Y.: Formation of Urinary and Bile-Duct Calculi in Animals Fed on Experimental Rations, in Saiki, T.: *Progress of Science of Nutrition in Japan*, League of Nations Health Organization, C. H. 523, Geneva, 1926, p. 369.

31. Stern, R.: *Arch. f. exper. Path. u. Pharmakol.* **112**:129, 1926; **131**:221, 1928.

32. Hansen, S.: *Acta chir. Scandinav.* **62**:483, 1927.

pigment stones without cholesterol content. This same procedure in hypocholesteremic rabbits, however, resulted in almost constant production of cholesterol crystal concretions.

Whitaker³³ reported soft masses in the gallbladders of four of sixteen cats after cutting and dilating the sphincter of the common bile duct.

Copher and Illingworth³⁴ repeated Whitaker's experiment on eighteen cats, but were unable to confirm his results. They also combined this procedure with the introduction of *B. coli*, staphylococci and streptococci in the gallbladders of cats, but found no cystitis or lithiasis even after several weeks. The same infection in dogs together with ligation of the common duct below the middle hepatic duct gave the same result. Cutting the sphincter in cats together with the introduction of many kinds of foreign bodies into the gallbladder, and in some cases with infection of the viscus, gave negative results. Nor could they produce stones by cauterization of either the mucosa or the serosa of the gallbladder with phenol after cutting the sphincter in cats.

Wilkie,³⁵ as did Rosenow, injected streptococci isolated from human gallbladders into the lumen of the gallbladder, into the wall of the gallbladder and intravenously in rabbits. The intravesical method gave negative results. The intramural method after three months produced an inflamed gallbladder, which contained pinhead-sized stones composed of large amounts of cholesterol but no calcium. The intravenous method was fruitless until, by repeated injections, chronic cholecystitis was produced, occasionally accompanied by the formation of cholesterol stones without trace of calcium. Injecting the streptococci intramurally with the cystic duct ligated produced stones in some cases. These stones contained a large amount of calcium as well as cholesterol. Repeated intravenous injection of the organisms with the cystic duct ligated resulted in the formation of stones composed mostly of calcium with but relatively little cholesterol.

Whitaker³⁶ stated that chemical examination of the masses that he produced in 1927 revealed them to be largely blood clots. He then reported masses resembling stones found in seven of nine cats, which he had produced by inducing stasis and hyperconcentration of bile through fasting and dehydration for from five to fifteen days while the animals were kept sleeping peacefully under barbital anesthesia. The stones consisted of numerous particles ranging in diameter from

33. Whitaker, L. R.: *J. A. M. A.* **88**:1542, 1927.

34. Copher, G. H., and Illingworth, C. F. W.: *Surg. Gynec. & Obst.* **46**:658, 1928.

35. Wilkie, A. L.: *Brit. J. Surg.* **15**:450, 1928.

36. Whitaker, L. R.: *Surg. Gynec. & Obst.* **48**:396, 1929.

that of a grain of dust up to 1 to 2 mm. and varying in consistency from semisolid to solid. No chemical analysis was reported.

Illingworth,³⁷ studying the "strawberry" gallbladder, reported the production of a large, semisolid concretion in the gallbladder of a single rabbit by feeding 0.2 Gm. of cholesterol daily for thirteen weeks and making an intramural injection of streptococci one week after the beginning of the feeding. No analysis or further description of the stone was reported.

Usuki³⁸ fed young rabbits a diet deficient in the fat-soluble vitamins for periods of from thirty-five to one hundred and seven days. Of the forty-three animals used, four developed numerous minute (millet seed) secretions.

Westphal and Gleichmann³⁹ narrowed the cystic duct in dogs for varying periods of time and combined this stasis with feeding of cholesterol and also with injections of animal charcoal and sodium chloride into the gallbladder. They succeeded in producing bilirubin concretions, usually of pinhead size, but in one case measuring up to 1 cm. in diameter. Microscopic examination of the bile from some of the animals fed cholesterol showed globules, presumably of cholesterol, radiating about the surface of minute pigment concretions. No cholesterol stones were found.

In summary, we find that, in the main, five methods have been used to produce calculi: introduction of foreign bodies into the gallbladder, production of stasis in the gallbladder, infection of the organ, induction of hypercholesteremia and production of a deficiency of vitamins. The use of foreign bodies even when combined with infection has resulted only in a coating of the bodies with a layer of pigment, and that only occasionally. Stasis in the gallbladder alone has given no results, except in one instance (Hansen) in which a pigment stone, without cholesterol content, was formed. Infection, alone and with stasis, has resulted in the formation of stones on many occasions. Often these stones were described as cholesterol stones, but in those instances in which analyses were made, the concretions were found to be either pigment stones, with or without calcium carbonate, or organic material consisting chiefly of tissue débris without appreciable cholesterol content. Cholesterol stones have been reported in a few instances in hypercholesteremic animals, and the character of the stones has been substantiated by analysis. Yet the animals in which positive results were shown numbered but a few of those used in each series, and other investigators have failed to confirm the results. Hypercholesteremia combined with stasis in the gallbladder has given no better success.

37. Illingworth, C. F. W.: *Brit. J. Surg.* **17**:203, 1929.

38. Usuki, K.: *Jap. J. Gastroenterol.* **1**:18, 1929.

39. Westphal, K., and Gleichmann, F.: *Ztschr. f. klin. Med.* **115**:329, 1931.

A few workers have reported finding minute cholesterol stones in vitamin-deficient animals. Too little information is available at this time to come to any conclusion concerning the application of these findings. Strangely, the combination of infection and hypercholesteremia has not been extensively tried. Illingworth found his concrection incidentally. The experimental work that I shall now describe concerns an attempt to produce cholesterol gallstones in rabbits by a combination of these two factors, continued high blood cholesterol plus infection of the gallbladder.

Data of Experiments

Rabbit	Length of Time Given 2 Injections of Cholesterol Weekly	Length of Time Survived Injections of Typhoid Bacilli	Cholecystitis	Gallbladder Content
1	6 wks.	(No injection)	0	Normal
2	7 wks.	(No injection)	0	Normal
3	2 mos.	(No injection)	0	Normal
4	2 mos.	(No injection)	0	Numerous hard pigment stones up to 1 mm. in diameter
5	2 mos.	(No injection)	0	Cholesterol crystals in the bile
6	4 mos.	(No injection)	0	Many minute pigment granules
7	5 mos.	(No injection)	0	Normal
8	(No cholesterol given)	5 mos.	Definite	Plug of cellular debris
9	(No cholesterol given)	16 mos.	Very definite	Lumen obliterated
10	3 mos.	12 mos.	0	Normal
11	5 mos.	12 mos.	0	Normal
12	5 mos.	5 mos.	0	Clear bile containing numerous minute, hard pigment granules
13	3 mos.	1 mo.	Definite	Cheesy, organic debris
14	4 mos.	3 mos.	Very definite	Organic debris and many hard pigment granules (2 x 1 mm.)
15	6 mos.	3 mos.	Slight	Cellular debris and microscopic hard pigment granules
16	5 mos.	7 mos.	Slight	Soft pigment clumps in bile
17	9 mos.	10 mos.	Slight	Many soft pigment clumps up to 1 mm. in diameter
18	2 mos.	2 mos.	Slight	Organic debris, small pigment granules, microscopic cholesterol crystals
19	6 mos.	11 mos.	Slight	Clumps of soft organic debris and cholesterol crystals in bile
20	5 mos.	3 mos.	Definite	Cellular debris and very numerous cholesterol crystals
21	6 mos.	10 mos.	Very definite	Heavy precipitate of cholesterol with 8 pigment clumps up to 3.5 mm. in diameter

METHOD

A 1 per cent watery emulsion of cholesterol was made according to the method described by Dewey.²¹ Healthy rabbits each received 10 cc. of this emulsion intraperitoneally twice a week for varying periods up to nine months. The blood cholesterol changes were followed and after the animals had become hypercholesteremic, the abdomen of each was opened, 1 cc. of bile was aspirated from the gallbladder, and about 1 cc. of a suspension of a twenty-four hour growth of typhoid bacilli was injected into the viscus. To prevent leakage into the peritoneal cavity, the needle was inserted into a tip of the liver, through the thin mesentery of the gallbladder, and thence through the wall of the gallbladder. The bile was aspirated, and the organisms injected through the same needle without removing it between the operations, so that only a single puncture was made. The abdomen was closed, and after two weeks' rest, the injections of cholesterol were

continued. The typhoid infection was produced from two to ten weeks after the injections of cholesterol were started, and these injections were continued for from five to thirty-two weeks after the infection. A few of the animals were killed. Some of them died from peritonitis resulting from the intraperitoneal injections. Those surviving the injections were left undisturbed to die of natural causes. These died from one to eight months after cessation of the injections.

OBSERVATIONS

Of the seven rabbits receiving injections of cholesterol only, four showed no biliary changes. Only one showed any cholesterol change in the gallbladder, and that was in the form of fine cholesterol crystals in the bile. Strangely, the bile of two rabbits contained black concretions, in one case of pinhead size, in the other measuring up to 1 mm. in diameter. These granules were irregular in outline and very hard. They contained no cholesterol, but were composed of calcium bilirubinate. The wall of the gallbladder was normal in all seven rabbits.

Both of the rabbits (8 and 9) receiving only the intravesical injection of typhoid bacilli showed marked chronic cholecystitis. The wall of the gallbladder was white, opaque and very thick. In the rabbit living five months after infection, the lumen of the viscus contained a plug of white, cellular debris and no bile. The gallbladder of the rabbit killed after sixteen months had a wall measuring 1 mm. in thickness, and the lumen was almost entirely obliterated.

Of the twelve rabbits receiving both the cholesterol and the typhoid injections, three showed a normal wall of the gallbladder with no evidence of infection. The bile was also normal in two of these, but in the third it showed pigment granules similar to those seen in the first group of animals. The other ten showed cholecystitis of varying degrees of intensity. In five there was slight thickening of the wall with a moderate degree of lymphocytic infiltration into the wall, as described in typhoid carriers (Mallory ⁴⁰). In two, this process was more severe, and there was some hypertrophy with fibrosis of the wall. The other two showed a very thick wall, composed largely of dense fibrous tissue. All of these ten showed changes in the bile. The bile in each case contained the organic debris seen in the rabbits receiving only the typhoid bacilli. The gallbladders of two of them also contained the hard pigment granules seen in the first group of rabbits receiving only the cholesterol. Six of the rabbits, however, showed further changes in the bile. In four, soft, brown clumps were found that did not resemble the small, hard, black granules of pigment, and at first these were thought to be masses of cholesterol. On analysis, however, the masses were shown to contain only traces of cholesterol and consisted largely of calcium bilirubinate. In two of these rabbits

40. Mallory, T. B., and Lawson, G. M.: *Am. J. Path.* **7**:71, 1931.



The gallbladder of rabbit 21. The rabbit received biweekly intraperitoneal injections of cholesterol for two months, followed by an intravesical injection of *B. typhosus* and a continuance of the injections of cholesterol for another four months. The animal was killed seven months later. Note the tremendously thickened wall of the gallbladder, the ten pigment concretions in the bile (one lies in the cystic duct, but does not occlude it) and the innumerable flecks of cholesterol present both in the bile and in the mucosa of the gallbladder.

there were also found in the bile large numbers of cholesterol crystals. Two other rabbits showed these crystals, but not the clumps of pigment. Rabbit 21 showed the condition illustrated in the accompanying photograph. The wall of the gallbladder was markedly thickened; the bile was filled with large flakes of yellowish cholesterol and contained ten soft concretions, the largest measuring irregularly 3.5 mm. in diameter. One of the smaller stones can be seen in the beginning of the cystic duct, where it was found lying loosely without occluding the duct. The bile was filled with precipitated cholesterol; yet an alcohol-ether extraction of the concretions showed only a trace of cholesterol on precipitation with digitonin.

COMMENT

Why two of seven hypercholesteremic rabbits should show pigment concretions in their gallbladders is unexplained. Hansen³² described similar findings. The literature contains no reference to spontaneous gallstones in rabbits. Only one of the seven showed cholesterol crystals in the bile. Of the ten hypercholesteremic rabbits with infected gallbladders, four showed cholesterol crystals in the bile, and two of these and two others showed changes in bile pigment not seen in the rabbits with noninfected gallbladders.

Although the method used failed to produce the desired result, cholesterol gallstones, yet it did result in precipitating out of the bile the elements making up gallstones. Many authors believe that cholesterol stones form by accumulation of precipitated cholesterol on bile pigment nuclei. Others feel that organic débris is the nidus. Four of the infected rabbits showed precipitation of cholesterol in the bile, six of them showed pigment nuclei, and all of them showed cellular débris in the bile. Pigment stones had formed, but no great quantity of cholesterol was adherent to the surfaces in the two cases in which both elements were present; nor had the cholesterol coated the cellular débris in the four cases in which the crystals and débris existed together. Whether some additional factor is necessary to lead to accumulation of the cholesterol on the nidus, or whether it is merely a question of greater time required for the growth of the stone, demands further investigation.

SUMMARY

A combination of hypercholesteremia, induced by intraperitoneal injections of a watery emulsion of cholesterol, and infection of the gallbladder, produced by direct cystic injection of *B. typhosus*, failed to lead to the formation of cholesterol gallstones in rabbits. Pigment calculi were formed in many of the gallbladders, and in some of the rabbits cholesterol crystals were found in the bile. In no instance, how-

ever, had any appreciable quantity of the cholesterol been incorporated in or incruited on the pigment stones.

The numerous methods used in attempts to produce gallstones experimentally are given in a review of the literature. Foreign bodies in the gallbladder have frequently become coated with pigment. Stasis alone has been unsuccessful, but when combined with infection has, on occasion, led to the formation of pigment stones often combined with carbonates. Cholesterol stones have been found in hypercholesteremic animals in a few instances, but these results have failed of confirmation. Recently, minute cholesterol stones have been reported in a few vitamin-deficient animals. Various combinations of the methods named have given no better results. No method has been found that will produce gallstones, especially of the cholesterol type, with any degree of certainty.

EFFECT OF VARIOUS ANESTHETICS ON THE CHOLESTEROL AND SUGAR CONTENT OF THE BLOOD

CORNELIUS A. HOSPERS, PH.D.

CHICAGO

In the course of experimentation on gallstone formation,¹ it was noted that animals under ether anesthesia showed a higher blood cholesterol value than they did preceding the anesthesia. The literature on this point was in some respects meager, and was conflicting. Therefore, experiments were carried out in an attempt to determine the amount and the extent of change in blood cholesterol under various anesthetics.

Bloor² reported a rise in blood fat (in which all the lipid constituents shared) of from 40 to 100 per cent in dogs under ether anesthesia. The rate of rise was most marked during the first hour. Using chloroform, Bloor found no rise in blood cholesterol unless the dog was previously stuffed with fat. Then there was a sharp rise. He noted an "after rise" from chloroform two or three days later. Both morphine and ethyl alcohol produced a slight or no increase in blood cholesterol, but did produce an after rise two or three days later. Reicher³ obtained a rise in blood cholesterol in dogs up to 300 per cent under ether anesthesia, and found the same results with chloroform. He reported morphine as producing a slight rise. According to Bürger and Schweisheimer,⁴ acute alcohol poisoning in the dog causes an increase in the blood cholesterol which appears only after the amount of alcohol in the blood decreases. There is no distinct lipemia at any time. Ducceschi⁵ subjected dogs to ether anesthesia for periods of from sixty to ninety minutes daily and found a marked rise in blood cholesterol. Similar experiments with chloroform showed a slight rise during the first two or three days, and then a decrease daily to below normal. Mann⁶ found an initial increase in blood cholesterol in dogs anesthetized with ether, followed by a decrease, after an hour or so of anes-

From the Department of Pathology, the University of Chicago.

1. Hospers, C. A.: The Experimental Production of Gallstones, with a Review of the Literature, *Arch. Path.* **14**:66 (July) 1932.

2. Bloor, W. R.: *J. Biol. Chem.* **19**:13, 1914.

3. Reicher, K.: *Ztschr. f. klin. Med.* **15**:235, 1908.

4. Bürger and Schweisheimer: *Ztschr. f. d. ges. exper. Med.* **5**:136, 1916.

5. Ducceschi, V.: *Arch. di farmacol. sper.* **27**:118, 1919.

6. Mann, F. C.: Some Bodily Changes During Anesthesia, *J. A. M. A.* **67**:172 (July 15) 1916.

sia, below the values taken as normal and then a gradual increase on further etherization. Mahler⁷ found that in man ether anesthesia produced a continuous rise in blood cholesterol proportional to the duration of the anesthesia. His longest period of anesthesia was one hundred minutes. He found no rise under nitrous oxide-oxygen anesthesia. Lattes⁸ found no rise from chloroform anesthesia. Manceau⁹ reported a slight rise in the blood cholesterol of monkeys from both chloroform and nitrous oxide anesthesia. Ginesty¹⁰ found the blood cholesterol lower after barbital anesthesia than it was beforehand. However, no determinations were made during narcosis. Recently, Gray¹¹ reported a definite rise in blood cholesterol after three weeks of repeated administrations of chloroform.

TECHNIC

Both dogs and rabbits were used in the experiments. Avoiding excitement as much as possible, the dogs were tied on their backs to an operating table. The femoral vein was exposed under 2 per cent procaine hydrochloride anesthesia. The dogs were kept quiet by petting and if excited were quieted for from twenty to forty minutes before the experiment proceeded. Approximately 1 cc. of blood was drawn from the exposed vein, twice in succession, and both samples were run in duplicate as normal blood cholesterol values. Ether anesthesia was instituted by the use of the ordinary closed dog mask attached to an ether bottle. The amount of ether given was kept constant, varying only with each dog initially, and in most instances producing complete relaxation without loss of the corneal reflex. One cubic centimeter of blood was drawn at intervals varying from every few minutes to every half hour. All samples were run in duplicate. The ether experiments in dogs covered periods of anesthesia up to ten hours. Rabbits were treated in the same manner except that they were placed in ordinary rabbit boxes and blood was drawn from the ear vein without local anesthesia. The ether was administered through a similar smaller apparatus. Anesthesia was continued for from four to six hours. Sackett's modification¹² of Bloor's method was used for the determination of the blood cholesterol values. This method requires but 0.25 cc. for each determination, so that the amounts of blood used eliminated any great hemorrhage factor. The total cholesterol of the whole blood was determined. In the experiments

7. Mahler, A.: *J. Biol. Chem.* **69**:653, 1926.

8. Lattes, L.: *Arch. f. exper. Path. u. Pharmacol.* **66**:132, 1911.

9. Manceau, P.: *Compt. rend. Soc. de biol.* **92**:1507, 1925.

10. Ginesty, Lassalle and Mériel: *Compt. rend. Soc. de biol.* **91**:1399, 1924.

11. Gray, S. H.: *J. Biol. Chem.* **87**:591, 1930.

12. Sackett, G. E.: *J. Biol. Chem.* **64**:203, 1925.

in which the blood sugar was measured also, an extra cubic centimeter of blood was drawn each time for the sugar determinations. Byrd's¹³ modification of the Folin method for the determination of blood sugar was employed, with the additional modification that 1 cc. of blood was used instead of 0.1 cc., and other solutions in corresponding amounts.

Experiments with chloroform were conducted in a similar manner, this anesthetic being substituted for ether in the ether bottle. Animals were anesthetized for as long as ten hours. Similar experiments were performed under nitrous oxide and ethylene anesthesia, using an apparatus to which was attached an ordinary dog mask containing an expiratory valve. Anesthesia was continued for from four to six hours with ethylene, and up to nine hours with nitrous oxide.

DATA

1. *Blood Cholesterol*.—Ether Anesthesia in Rabbits: The experiment was performed on six rabbits. The blood cholesterol values taken as normal varied from 60 to 96 mg. per hundred cubic centimeters of blood. Blood was drawn every fifteen or thirty minutes after the initiation of ether anesthesia. The changes in blood cholesterol values are shown in chart 1, on which is represented the amount of change from the normal value for each rabbit. The results of three typical experiments are charted, together with an average curve of the changes in all six rabbits averaged for each time period. It will be noted that the blood cholesterol began to rise rapidly with the advent of anesthesia and continued to do so for from forty-five to sixty minutes. Continued anesthesia was not accompanied by a further rise, but the hypercholesteremia began to decrease somewhat less rapidly than it rose, and then more slowly continued to drop toward normal, reaching it after about four hours of anesthesia. The peak of hypercholesteremia, then, is seen to occur after approximately one hour of anesthesia, the amount varying from 38 to 100 mg. above the normal.

Ether Anesthesia in Dogs: Ten dogs were used. The values taken as normal ranged between 130 and 150 mg. per hundred cubic centimeters of blood. In five dogs ether anesthesia was slowly induced to such a degree as to produce complete relaxation but not to such an extent as to abolish the corneal reflex (surgical anesthesia). The amount of ether given was then kept constant. Blood was drawn as before. Three typical examples of the blood cholesterol changes are illustrated on chart 2a; the values represent the changes from the normal as determined for each dog. The blood cholesterol was found to fluctuate more than in rabbits, but a similar change is seen. A peak in the hypercholesteremia occurs after from thirty to ninety minutes

13. Byrd, T. L.: J. Lab. & Clin. Med. **2**:67, 1925.

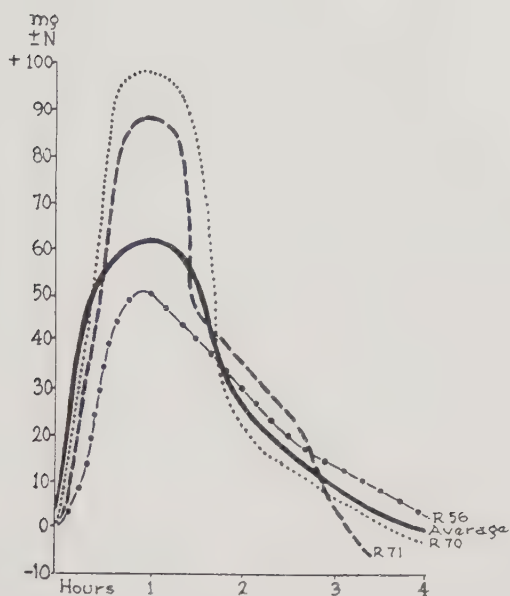


Chart 1.—Changes in the blood cholesterol of rabbits under ether anesthesia.

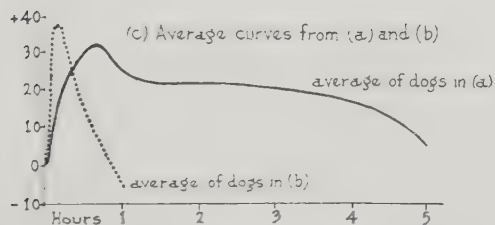
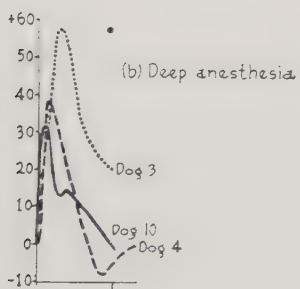
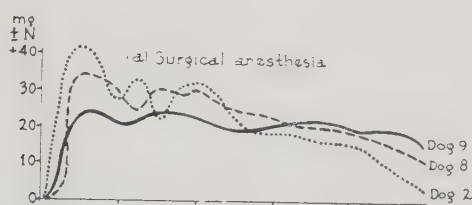


Chart 2.—Changes in the blood cholesterol of dogs under ether anesthesia.

of anesthesia; then a gradual return toward the normal, approaching it after about five hours of anesthesia. This is best illustrated by plotting a curve from the average of all five dogs for each time period, chart 2c. The change from normal is less in dogs than in rabbits, the highest point of hypercholesteremia varying from 18 to 42 mg. The blood cholesterol was found to be sensitive to amounts of ether given. If, at any point during the experiment, the amount of ether was increased, a rise in the blood cholesterol followed. This is illustrated in chart 3 by the terminal rise in dogs due to an increase in the rate of anesthesia to kill the animals, and by the secondary rise in blood cholesterol after an increase in the depth of anesthesia in both dogs and rabbits. Other examples are included in the later section on changes in the blood sugar.

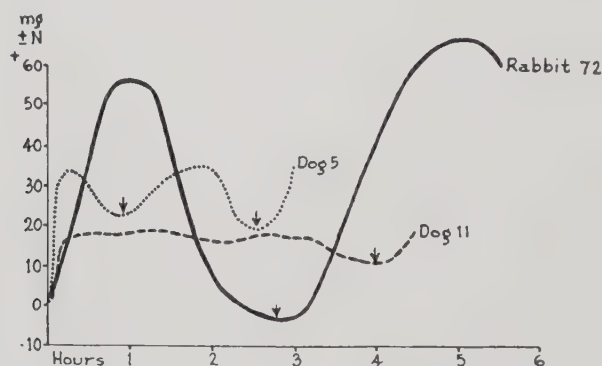


Chart 3.—Changes in the blood cholesterol due to increasing the rate of anesthesia (arrows indicate point of increase).

In three dogs the anesthesia was given much more rapidly, larger amounts of ether being given to produce rapid and deep anesthesia. Chart 2b shows a more rapid rise than in surgical anesthesia, a peak being reached in from five to ten minutes, and a more rapid drop toward normal, which is reached after about an hour of anesthesia. The average of these dogs shows a higher peak as well as a more rapid change.

Two dogs subjected to smaller quantities of anesthetic, so that only partial relaxation was obtained and reflexes were very active, showed a much lower peak and much more fluctuation.

Chloroform Anesthesia: Eight dogs were used. Again three typical experiments are illustrated (chart 4), together with the composite curve averaged from the changes in all eight dogs. The values taken as normal ranged from 127 to 200 mg. per hundred cubic centimeters of blood, some of which are probably higher than the true normal for the dogs. If determinations were made during the first

few minutes of anesthesia, the blood cholesterol was found to fluctuate somewhat, even falling below the values taken as normal, especially if it was rather high. The curves are plotted, using fifteen minutes after the introduction of anesthesia as the initial point of change. A gradual, continuous rise in the blood cholesterol occurred, proportional to the length of anesthesia. Three dogs allowed to recover after the experiment showed a continuing rise in blood cholesterol for at least twenty-

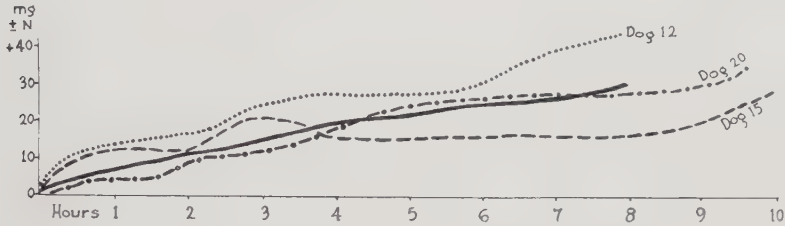


Chart 4.—Changes in the blood cholesterol of dogs under chloroform anesthesia. The heavy black line shows the average for eight dogs.

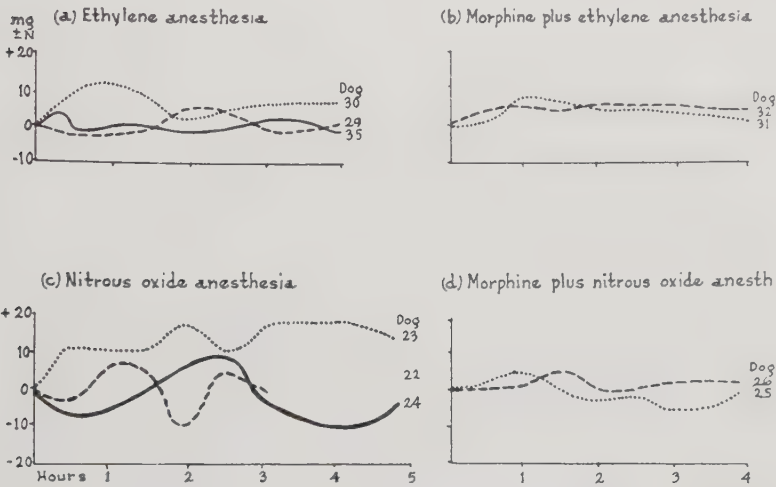


Chart 5.—Changes in the blood cholesterol of dogs under (a) ethylene, (b) morphine plus ethylene, (c) nitrous oxide and (d) morphine plus nitrous oxide anesthesia.

four hours. After a week, however, the values were down to normal limits. Table 1 gives the essential figures for one of these experiments.

Ethylene and Nitrous Oxide Anesthesia: Ethylene-oxygen anesthesia alone was found to be rather poor for dogs. It was somewhat difficult to secure a state of good relaxation, and a concentration of 95 per cent ethylene to 5 per cent oxygen was required. Five dogs

were used. The results in three are charted (chart 5) for the first four hours. There was no great change in the blood cholesterol at any period, but a moderate fluctuation was constant. Two dogs given 30 mg. of morphine sulphate subcutaneously about half an hour before the experiment showed much less fluctuation (chart 5b).

Nitrous oxide-oxygen anesthesia was found to be even less satisfactory as an anesthetic for dogs; 95 per cent nitrous oxide to 5 per cent oxygen was used. It was difficult to abolish voluntary movements

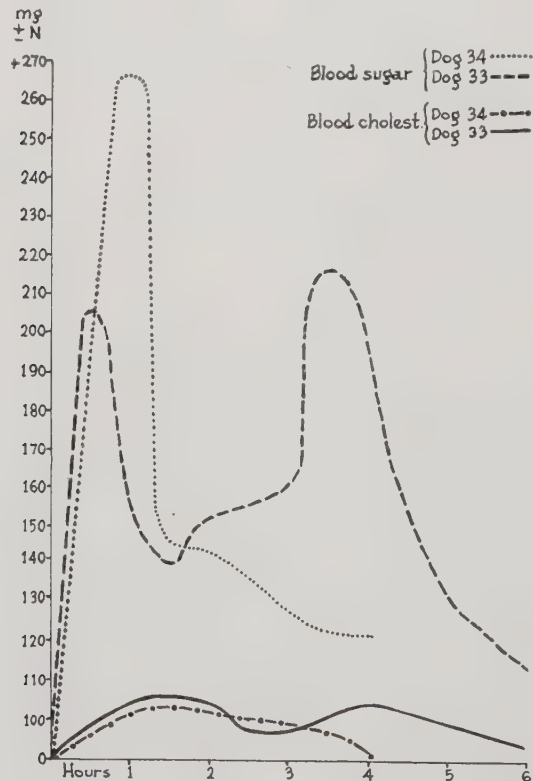


Chart 6.—Changes in the blood sugar and blood cholesterol under ether anesthesia.

TABLE 1.—Changes in Blood Cholesterol in Chloroform Anesthesia (Dog 16) *

		Cholesterol, Mg. per 100 Cc.
5/12/31: 1:10 p.m.	Normal blood cholesterol.....	216
7:45 p.m.	After 6½ hours of chloroform anesthesia.....	240
5/13/31: 7:30 p.m.	Twenty-four hours after anesthesia.....	253
5/19/31: 2:25 p.m.	One week after anesthesia.....	206

* A black male terrier; weight, 10.9 Kg.

and almost impossible to secure complete relaxation. The fluctuations were greater than with ethylene (chart 5c), but here, too, there was no great or constant change in the blood cholesterol. Morphine, given beforehand, diminished the fluctuation markedly and resulted in a minimal change from the normal (chart 5d).

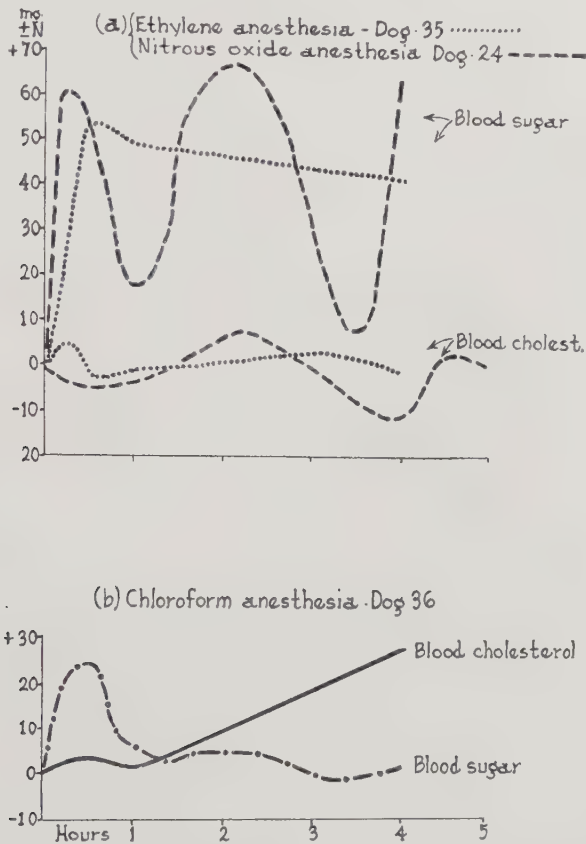


Chart 7.—Changes in the blood sugar and blood cholesterol under (a) ethylene and nitrous oxide anesthesia, and (b) chloroform anesthesia.

Excitement: As has been indicated, some of the changes in blood cholesterol were thought to be due to something other than the anesthetic. On numerous occasions blood drawn when the animal was excited from tying, etc., showed higher cholesterol values than succeeding samples taken after the dog had been quiet for some twenty-five minutes. These changes are shown in table 2. Table 3 shows the changes taking place in a dog before and after excitement from playing and fighting. Excitement was accompanied by a hypercholesteremia

TABLE 2.—*Effect of Excitement on the Blood Cholesterol of Dogs*

Dog	Time		Cholesterol, Mg. per 100 Cc.	Rise, Mg. per 100 Cc.
4	1:30 p.m.	Blood drawn from quiet dog.....	148	12
	1:55 p.m.	Dog excited by presence of cat; then tied down without any attempt at quieting.....	160	
18	10:40 a.m.	Dog tied to table, causing excitement; blood drawn immediately afterward	210	10
	11:55 a.m.	Blood drawn after quieting dog since 10:40.....	200	
28	9:00 a.m.	Dog tied to table; excited; blood drawn.....	213	11
	9:25 a.m.	Blood drawn after quieting dog.....	202	

TABLE 3.—*Effect of Excitement on Blood Sugar and Blood Cholesterol in Dog 37**

Date	Time		Sugar, Mg. per 100 Cc.	Change, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.	Change, Mg. per 100 Cc.
12/4/31	9:05 a.m.	After playing excitedly for about one hour	141	29	157	10
	10:15 a.m.	After lying quiet for one hour...	112		147	
12/5/31	1:00 p.m.	After spending a morning alone in a quiet room.....	97	55	142	17
	2:30 p.m.	After a fight with another dog, preceded by playing for ½ hour	152		159	
12/7/31	6:50 p.m.	After being alone in a quiet room all afternoon.....	105	32	139	9
	7:30 p.m.	Following 30 min. of tumbling play, pulling, jumping, etc. ...	137		148	
12/8/31	9:30 a.m.	Following 20 min. of play.....	131	26	153	6
	11:50 a.m.	After quiet of two hours.....	105		147	

* A pet terrier, very lively but trained to obey. The dog was fed on a diet of meat and bread once a day, in the evening after the completion of the experimentation.

TABLE 4.—*Effect of Excitement on Blood Cholesterol of Rabbits*

Rabbit	Time (10/6/31)		Cholesterol, Mg. per 100 Cc.	Change, Mg. per 100 Cc.
74	2:05 p.m.	Put in box; rather quiet		
	2:15 p.m.	Blood taken	85	
	2:27 p.m.	Blood taken	85	
	2:36 p.m.	Blood taken	83	
		(Average, 84)		
	2:40 p.m.	Removed from box; placed before dogs, chased about, rolled onto back, rolled over and over, etc., for 20 minutes		
	3:00 p.m.	Blood taken	93	8 (rise)
75	3:05 p.m.	Chased about cage, run about floor, rolled, etc., for 15 minutes		
	3:20 p.m.	Blood taken	93	
	3:25 p.m.	Blood taken	93	
	4:20 p.m.	Blood taken	88	
	4:40 p.m.	Blood taken	85	8 (drop)
8-76	4:30 p.m.	Taken from cage and put in box quietly		
	4:45 p.m.	Blood taken	80	
	5:00 p.m.	Blood taken	76	
		(Average, 78)		
	5:05 p.m.	Taken from box; thrown into air, chased (not caught) by a dog, rolled over and over on floor many times, etc., until 5:35 p.m.		
	5:40 p.m.	Blood taken	89	
	5:50 p.m.	Blood taken	88	10 (rise)

averaging about 10 mg. above the normal. Table 4 illustrates similar changes in rabbits with relation to excitement.

2. *Blood Sugar*.—Ether Anesthesia: On two dogs the changes in blood sugar were followed with the blood cholesterol changes. Chart 6 illustrates the changes in the two blood constituents. The blood sugar rose rapidly to reach a peak within the first hour of anesthesia, and then gradually fell toward normal. The blood cholesterol, changing much less, showed a slightly slower but similar response. Dog 33 also illustrates the effect of changes in the depth of anesthesia. After one and a half hours of anesthesia, the amount of ether being given was slightly increased. At the end of three hours of anesthesia, the ether bottle was refilled, resulting in a temporary increase in the amount of anesthesia given. The corresponding rise in both blood sugar and blood cholesterol values is apparent.

Other Anesthetics: Under chloroform anesthesia the change in blood sugar was similar to that under ether: a rapid rise and a gradual fall to normal, in spite of the fact that the blood cholesterol continued to rise (chart 7*b*). Under ethylene and nitrous oxide anesthesia the blood sugar showed the same rapid rise, with subsequent fluctuation about this elevated point (chart 7*a*).

Excitement: Table 3 demonstrates the rise in blood sugar after excitement not related to anesthesia.

COMMENT

Mann⁶ suggested that the initial rise in blood cholesterol under ether anesthesia might be due to excitement. Excitement must play some part in the hypercholesteremia. Lyons¹⁴ has recently reported an emotional hypercholesteremia in cats: an increase in blood cholesterol of from 25 to 30 per cent after from twenty to forty minutes of excitement. This change was absent after the removal of the sympathetic nervous system. But this factor does not seem to be entirely responsible. The excitement stage under all the anesthetics used was similar, yet the rise of blood cholesterol under ether was greater than under the others. Then, too, excitement without anesthesia produced less change than occurred with the anesthesia. Dog 11 (chart 3) received barbital before the ether anesthesia. Excitement was eliminated, and the rise was less marked but not absent. Dog 4 (table 2) was anesthetized immediately after the excitement experiment, and by comparison with chart 2*b* it will be seen that ether anesthesia was accompanied by a greater hypercholesteremia than occurred with excitement in the same dog.

14. Lyons, C.: *Am. J. Physiol.* **98**:156, 1931.

The fact that blood sugar rises under anesthesia is well established. All authors do not agree as to the type of change. Macleod¹⁵ thought that the changes in the dextrose content of the blood from anesthesia were slight. Ross and McGuigan¹⁶ stated the increase in blood sugar to be general and consistent, and demonstrated a continuance of the high sugar content of blood after the cessation of anesthesia. Their periods of anesthesia lasted two hours. Examination of their tables shows some values still rising after two hours, but others have already reached a peak at one hour and are lower after two hours. The after rise may be associated with excitement as consciousness returns. Sansum and Woodyatt,¹⁷ working with phlorizinized dogs, found an initial increase in blood sugar with ether and with chloroform, followed by a fall. Nitrous oxide failed to produce such changes. Mahler⁷ reported a continuous rise in blood sugar under ether anesthesia for periods of one hundred minutes. Mackay and Dyke¹⁸ found a rapid initial rise, with a gradual return to the original level. Trout¹⁹ has reported a postoperative increase in blood sugar after twenty minutes of ethylene anesthesia.

The cause of the hyperglycemia is still more disputed. In 1910, Macleod and Pearce²⁰ demonstrated a reduction in the glycogen content of the liver following ether anesthesia. Shaffer²¹ thought that the rise in sugar was due to excitement and asphyxia, but King, Moyle and Haupt²² had previously eliminated asphyxia by producing the anesthesia by intravenous injection of ether, and still obtained a hyperglycemia. Ross and McGuigan¹⁶ felt that the effect was not due to excitement or asphyxia but to the ether itself acting as an activator on liver glycogen, permitting the action of diastase on it. However, Stewart and Rogoff,²³ working with adrenalectomized rabbits, found a greater increase in blood sugar from asphyxia superimposed on ether anesthesia than from the anesthesia alone. Ross and Davis²⁴ suggested that ether hyperglycemia might be due to depression of the internal secretion of the pancreas, and Mahler⁷ used this theory to explain the absence of the rise in blood sugar on the subcutaneous injection of

15. Macleod, J. J. R.: *Diabetes: Its Pathological Physiology*, New York, E. Arnold, 1913, p. 187.

16. Ross, E. L., and McGuigan, H.: *J. Biol. Chem.* **22**:407, 1915.

17. Sansum and Woodyatt: *J. Biol. Chem.* **21**:1, 1915.

18. Mackay, R. L., and Dyke, S. C.: *Brit. J. Anaesth.* **6**:61, 1928.

19. Trout, H. H.: *Anesth. & Analg.* **8**:269, 1929.

20. Macleod, J. J. R., and Pearce, R. G.: *Am. J. Physiol.* **27**:341, 1910-1911.

21. Shaffer, P. A.: *J. Biol. Chem.* **19**:297, 1914.

22. King, Moyle and Haupt: *J. Exper. Med.* **16**:178, 1912.

23. Stewart, G. N., and Rogoff, J. M.: *J. Pharmacol. & Exper. Therap.* **15**:238, 1920.

24. Ross, E. L., and Davis, L. H.: *Am. J. Physiol.* **53**:391, 1920.

insulin previous to anesthesia. Fuss²⁵ believed ether hyperglycemia due to mobilization of sugar from the liver. Rosenthal and Bourne²⁶ found that hepatic function was diminished after anesthesia, and Cantarow and Gehret²⁷ cited clinical material demonstrating that patients with hepatic disease do not show a rise in blood sugar under ether as other patients do.

It seems most probable that the hyperglycemia is due to increased hepatic glycogenolysis. Ether itself must play a part in this process, since it is accompanied by a rise so markedly greater than that of excitement alone. The hyperglycemia occurring in chloroform, ethylene and nitrous oxide anesthesia is much less in amount and may very well be due to excitement, which alone can lead to a rise in blood sugar equal to that from these anesthetics. It has been shown that in ether anesthesia the changes in blood sugar and in blood cholesterol tend to parallel each other. Then, if the change in blood sugar is due to increased mobilization from the liver, may not at least part of the increased amount of cholesterol in the blood have the same origin?

Insulin prevents the rise of both these blood constituents under anesthesia. Remesow and his co-workers²⁸ have recently found that while cholesterol leads to glycogenolysis in the liver, cholesterol plus insulin results in the storage of glycogen in the liver. In anesthesia the effect may be due, as stated, to a suppression of the internal secretory function of the pancreas, and if insulin is given artificially it may nullify the glycogenolysis by its power to increase storage.

However, the hypercholesteremia cannot be attributed entirely to loss of lipid from the liver. Other organs also release cholesterol into the blood. Manceau⁹ reported a considerable diminution in the cholesterol content of the adrenal gland from chloroform anesthesia. After the administration of ethyl alcohol to dogs over a period of days, Ducceschi²⁹ found a constant decrease in the cholesterol content of the adrenal gland averaging 40 per cent, and sometimes a decrease in other organs, such as the testis.

The gradual drop in hypercholesteremia after an hour's anesthesia may be due to the gradual lowering of the amount of cholesterol easily separated from the tissues. Since ether does not produce much toxic

25. Fuss, H.: *Klin. Wchnschr.* **9**:410, 1930.

26. Rosenthal, S. M., and Bourne, W.: *The Effect of Anesthetics on Hepatic Function*, *J. A. M. A.* **90**:377 (Feb. 4) 1928.

27. Cantarow, A., and Gehret, A. M.: *Ether Hyperglycemia*, *J. A. M. A.* **96**:939 (March 2) 1931.

28. Remesow and Matrossowitsch: *Ztschr. f. d. ges. exper. Med.* **77**:67, 1931. Remesow, Matrossowitsch and Sepalowa: *ibid.* **77**:100, 1931.

29. Ducceschi, V.: *Arch. ital. de biol.* **70**:93, 1920.

degeneration, cholesterol from that source is not liberated, and as removable cholesterol diminishes in the tissues, the blood cholesterol falls to or below normal.

The change in the cholesterol content in chloroform anesthesia is more easily explained. The initial changes are of the same magnitude as the changes with excitement, and may be attributed to that factor (dog 18, table 2). The gradual but continuous rise in blood cholesterol with continuous chloroform anesthesia is, perhaps, the result of toxic degeneration of the liver cells with liberation of the lipid substances, increasing in amount as the destruction of the tissue proceeds. In 1909, Howland and Richards³⁰ suggested this as the cause of the after rise of blood lipoids following chloroform poisoning.

The fluctuations in blood cholesterol found throughout ethylene and nitrous oxide anesthesia are of the same magnitude as those produced by excitement and, as with the hyperglycemia, may be attributed entirely to it, especially since these gases fail to produce a good anesthetic state in the dogs. The diminution of the fluctuations when morphine precedes the anesthesia also indicates that excitement is the main factor in the changes.

Although it was known that ether anesthesia is attended by a hypercholesteremia, Mann⁶ alone noted that this is temporary and is followed by a drop to normal under continued anesthesia. He attributed the rise to excitement. This report gives evidence that although excitement is a factor, ether itself is responsible for a transient but not continued hypercholesteremia, and that the more generally known changes in blood sugar are proportional to the lipid changes. Chloroform anesthesia has been reported as producing no rise (Bloor² and Lattes⁸), a slight rise (Ducceschi⁵ and Manceau⁹) and a great rise (Reicher³) in acute experiments. An after rise has been found constantly. The experiments in this paper show an absence of immediate change but a gradual, continuous rise proportional to the length of anesthesia. The after rise is also noted. Under nitrous oxide anesthesia, Manceau⁹ found a slight rise in the blood cholesterol of monkeys. According to Mahler,⁷ patients previously given morphine showed no rise in blood cholesterol under nitrous oxide. The data here presented show no changes due to nitrous oxide. An investigation of the literature revealed no studies of the blood cholesterol under ethylene anesthesia. This report indicates that ethylene has no effect on the blood cholesterol.

The differences in previously reported results on changes in blood sugar and blood cholesterol under various anesthetics can be attributed largely to a factor seldom considered, that of excitement. Shaffer²¹ and Mann⁶ suggested that excitement affected the blood sugar, and Lyons¹⁴

30. Howland, J., and Richards, J.: *J. Exper. Med.* **11**:344, 1909.

showed for the first time (1931) that emotion disturbed the blood cholesterol. The experiments reported in this paper demonstrate that excitement in rabbits and dogs causes both a hyperglycemia and a hypercholesteremia, and that the excitement seems to be responsible for the changes in blood sugar and the acute changes in blood cholesterol under chloroform, nitrous oxide and ethylene anesthetics.

Chloroform and ether anesthesia disturb the lipid metabolism, and ether affects the carbohydrate metabolism as well. Nitrous oxide and ethylene—of more import because of its wider applicability—disturb neither lipid nor carbohydrate metabolism, and seem therefore to be more physiologic anesthetics.

SUMMARY

1. Continuous ether anesthesia in dogs and rabbits is accompanied by a hypercholesteremia that, in ordinary surgical anesthesia, reaches a peak in from sixty to ninety minutes and thereafter gradually diminishes in amount, returning to approximately normal limits in from four to five hours.

2. Continuous chloroform anesthesia is accompanied by slight immediate change but by a gradual and continuous rise in the blood cholesterol proportional to the duration of anesthesia (up to ten hours).

3. Ethylene and nitrous oxide anesthetics are accompanied by no changes in the blood cholesterol other than those explainable by excitement and imperfect anesthesia.

4. Excitement is accompanied by a hypercholesteremia. This factor increases, but is not entirely responsible for the change found under ether anesthesia. It seems to be responsible for initial changes under chloroform and for the irregularity in the blood cholesterol values under ethylene and nitrous oxide.

5. Ether anesthesia is also accompanied by a hyperglycemia that frequently tends to be proportional to the hypercholesteremia. Chloroform, ethylene and nitrous oxide anesthesia are accompanied by a hyperglycemia, as is excitement without anesthesia.

6. Ethylene and nitrous oxide, as anesthetics, seem to be more physiologic in their action than ether and chloroform.

